



Comparison between concentrations of amphotericin B in infected lung lesion and in uninfected lung tissue in a patient treated with liposomal amphotericin B (AmBisome)[☆]

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SUMMARY

Generally, the primary lesion of a mold infection is in the airway, an extravascular site. Therefore, the antifungal drug concentration at the actual tissue lesion of a mold infection is as important as in the blood compartment. Although our antifungal armamentarium has expanded recently, polyenes are still often needed in clinical practice because of their potent fungicidal activity and the rarity of resistance. Nevertheless, the distribution of amphotericin B (AmB) in infected lung tissue has not yet been evaluated. Using high-performance liquid chromatography analysis, we determined the concentrations of AmB in plasma and infected and uninfected tissues of resected lung simultaneously, in a patient with pulmonary aspergillosis treated with liposomal amphotericin B (L-AmB). The AmB concentration in the infected lesion of the lung was approximately 5.2 times higher than that in plasma and 3.7 times higher than in uninfected lung tissue. L-AmB accumulated in the infected lesion of the lung at a higher concentration. Although our data are from only one patient, they may be useful in helping to develop better strategies for the use of L-AmB against pulmonary fungal infections.

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1. Introduction

The lung is the most common site of aspergillosis, a life-threatening disease with a dramatically increasing prevalence.¹ Recently, we have been able to formulate several antifungal strategies with new azoles and echinocandins, but polyenes remain important in clinical practice because of their potent fungicidal activity.² However, the use of conventional amphotericin B deoxycholate (CAB) is limited because it often causes renal failure, hypokalemia, or infusion-related reactions.

Liposomal amphotericin B (L-AmB, AmBisome[®]; Gilead Sciences, Sandimas, CA, USA) is a lipid formulation of amphotericin

B (AmB): a preparation of small, unilamellar liposomes with an average size of 60–100 nm.³ This drug exerts uniform fungicidal activity and its toxicity-related events are significantly reduced.² The improved adverse drug event profile and the contribution to survival advantages are superior to those of CAB.^{2,4}

The primary portal of entry of *Aspergillus* is the airway, an extravascular site. Therefore, the antifungal drug concentration at this actual site of fungal infection is as important as that in the blood compartment. Smith et al. conjectured that more drug is delivered to the mouse lung when it is infected with *Aspergillus fumigatus*.⁵ It was presumed that AmB would accumulate more in inflammatory lung tissue than at a normal lung site. However, to our knowledge, no human data have been reported regarding drug concentrations in infected and uninfected lung tissues in patients treated with known doses of L-AmB.

In this report we describe a patient with pulmonary aspergillosis who was treated with L-AmB. We were able to evaluate the concentrations of AmB in plasma, infected lung lesion, and uninfected lung site at the same time.

[☆] This study was presented in part, in Japanese, with the title "A case of pulmonary aspergillosis associated with advanced esophageal cancer", at a poster session of the 83rd Annual Meeting of the Japanese Association for Infectious Diseases, held in Tokyo, April 23–24, 2009.

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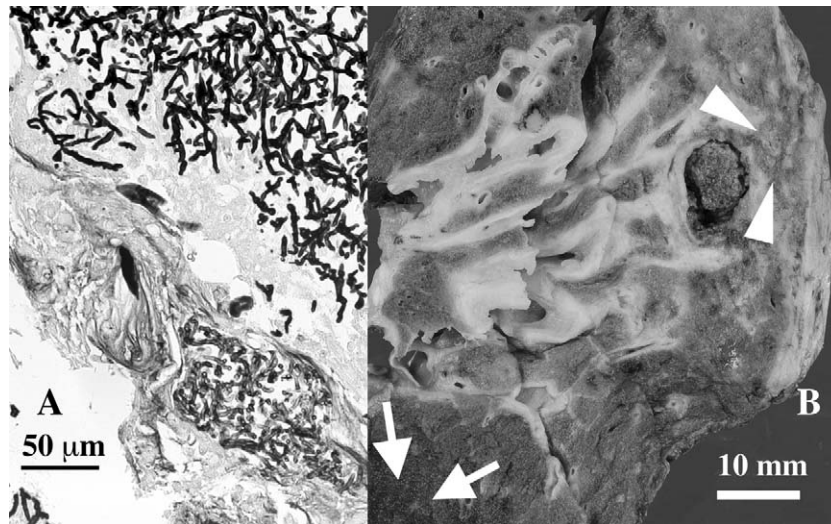


Fig. 1. The pulmonary aspergillosis lesion in the patient. (A) A trans-bronchial lung biopsy was performed from the right upper lobe. Thin separate hyphae showing dichotomous branching are seen; GMS stain ($\times 400$). (B) Two tissue samples were obtained from the resected lung. A sample was removed from the infected lesion (arrowheads), and another was cut from the uninfected area (arrows).

2. Case report

A 50-year-old male Japanese patient with advanced esophageal cancer was referred to our hospital for anticancer chemotherapy. He had multiple metastases, including lung and bone, and could not be treated with surgery. Full examinations including bronchoscopy were performed before chemotherapy, and a pulmonary fungal infection in the right upper lobe of the lung was revealed. Pathologically, brown-colored, thin separate hyphae showing dichotomous branching and crystals of calcium oxalate were observed. Using an in situ hybridization technique,⁶ this fungus was revealed to be *Aspergillus* sp. From these findings, the causative pathogen was determined to be *A. niger* (Fig. 1A). Cultures of sputa and the biopsied specimen were negative. Renal and hepatic functions were not impaired. The patient expressed a strong desire to begin anticancer drugs; therefore, we started him on voriconazole (400 mg/day) concurrently with his cancer treatment.

After receiving the anticancer drugs, his body temperature rose sharply (39.5°C), and there were increases in his white blood cell count ($15.2 \times 10^9/\text{l}$) and C-reactive protein value (7.6 mg/dl). A chest computed tomography scan revealed deterioration of the aspergillosis. Based on these findings, the antifungal drug was changed to L-AmB (150 mg/day (2.8 mg/kg) for the first 4 days, subsequently 250 mg/day (4.7 mg/kg)), and remission was quickly achieved.

Twenty-two days after starting L-AmB, the patient again developed a fever (39.2°C), and radiological findings indicated ingravescence of aspergillosis. Micafungin was added, but a drug rash forced its discontinuation. Flucytosine (5-FC; 10 g/day) was then added to the current antifungal therapy as a workaround. Fortunately, he soon became afebrile with this antifungal combination therapy.

In accordance with the patient's unwavering demand for continuance of his anticancer therapy, a right upper lobectomy was performed to remove the infectious focus. The total amount of AmB administered up until the surgery was 11 850 mg.

With written consent provided by the patient, we determined the concentrations of AmB in plasma and lung tissues. Blood and lung tissues were sampled during the operation. Blood samples had been taken just before L-AmB administration, immediately after administration was finished, and at the time of the right

upper lobe resection. The duration of L-AmB infusion was 3 h. Plasma was separated by centrifugation. Two samples of lung tissue (approximately 1 g, wet weight) were obtained, one from an infected lesion and one from a distant, uninfected site that was assessed macroscopically (Fig. 1B). Later, we examined adjacent regions of each sample pathologically for confirmation. Plasma and tissue samples were frozen at -80°C until the measurement of AmB concentration.

The concentrations of the drug were evaluated by a high-performance liquid chromatography (HPLC) method as previously described by Takemoto et al.,⁷ with some modification. The internal standard solution was obtained by dissolving 1-amino-4-nitronaphthalene (Sigma-Aldrich, Tokyo, Japan) in methanol at 30 mg/l. The plasma sample (150 μl) was extracted by the addition of internal standard solution (150 μl) and methanol (500 μl). Methanol was added for protein precipitation. After vortexing for 1 min and centrifuging at 10 000 g for 10 min, 200 μl of supernatants was collected and evaporated. The residue was re-suspended in 100 μl of acetonitrile–2.5 mM EDTA (pH 5.0) 1:2 v/v and vortexed for 30 s. This sample, containing the total amount of AmB, was submitted to HPLC analysis (50 μl injected).

Lung tissue sample homogenization was carried out according to the method described by Vogelsinger et al.,⁸ with some modification. Each lung tissue sample (0.15 g) supplemented with methanol (1 ml) was homogenized in a homogenizer (IKA T10 basic ULTRA-TURRAX, IKA Japan Co., Ltd, Yamatokoriyama, Japan) at 20 450 rpm. The tissue samples were used for preliminary and other studies as well, and therefore not all of them could be used repeatedly for evaluating the concentration. The homogenates were extracted by a method similar to the plasma extraction described above and submitted to HPLC analysis (50 μl injected).

The HPLC system consisted of LC-20AT solvent delivery pump systems and an SPD-10A_{vp} detector (Shimadzu Co., Kyoto, Japan). The supernatants were separated on a CAPCELL PAK C₁₈ SG120 reverse phase column (4.6 $\phi \times 250$ mm; Shiseido Co., Tokyo, Japan). The mobile phase consisted of acetonitrile–2.5 mM EDTA (pH 5.0) 1:2 v/v at a flow rate of 1.0 ml/min. Under these conditions, AmB and the internal standard were demonstrated at retention times of approximately 13 and 20 min, respectively, and were detected at 405 nm. The AmB concentration was determined from the ratio between the area of AmB and that of the internal standard. Standard curve samples were prepared with AmB

Table 1
Plasma and tissue concentrations of amphotericin B

	Trough	End of infusion	Resection of lung
Plasma (mg/l)	32.8	60.3	60.8
Uninfected lung (μg/g)	N/A	N/A	86.2
Infected lung (μg/g)	N/A	N/A	316.0

(purchased from Sigma Chemical Co., St Louis, Mo, USA); AmB was dissolved in methanol/dimethylsulfoxide 1:1 v/v and serially diluted with methanol. The lower limit of detection in plasma was 0.1 mg/l and in lung tissue was 0.1 μg/g.

3. Results and discussion

The trough concentration of AmB in plasma was 32.8 mg/l, and the concentration at the end of drug infusion was 60.3 mg/l. At the time of lung lobe resection, the plasma concentration of the drug was 60.8 mg/l (Table 1). These plasma concentration results are in line with those reported by Walsh et al.⁹ In contrast, the AmB concentration in the infected lung lesion was 316.0 μg/g. The concentration of AmB in the uninfected lung was 86.2 μg/g (Table 1). No metastasizing tumor was found in the resected lung tissue.

To the best of our knowledge, this is the first report of a comparison of drug concentrations in infected and uninfected tissue in a patient treated with a specific dose of L-AmB. Paterson et al., using HPLC, reported AmB levels in lung tissue infected with *Aspergillus* sp. and in the background lung tissue of a patient.¹⁰ This patient was treated with AmB for 28 days and died from invasive aspergillosis. The concentrations in the infected lesion and normal background lung were 0.1 and 0.67 μg/g, respectively. However, this study did not mention the type of drug formulation used (L-AmB or CAB) or the dose given to the patient. In contrast, we have documented AmB concentrations in infected and uninfected sites of the same lung of a patient treated with known doses of L-AmB.

Collette et al. estimated that only approximately 18–40% of tissue-bound AmB is bioactive.¹¹ Thus, the increased antifungal drug concentration at the actual lesion of a fungal infection, such as in pulmonary aspergillosis, might be important. Moreover, the lung architecture is mostly extravascular, and great emphasis has recently been placed on antifungal activity at the tissue site of infection.

The AmB concentration in infected lung tissue was approximately 5.2 times higher than that in plasma, whilst that in uninfected lung tissue was only 1.4 times higher. In general, L-AmB is eliminated from the circulation primarily by the organs of the reticulo-endothelial system, such as the liver and spleen.^{5,12} The drug is effective at relatively low doses because of its mechanism of cellular delivery: tissue phospholipases cleave AmB, and the drug then enters macrophages and fungus-infected cells.¹³ Smith et al., by immunohistochemical procedure, showed that L-AmB given to mice was localized primarily within macrophages.⁵ Takemoto et al. demonstrated that L-AmB itself was localized at the infected sites in the lung of a mouse model of pulmonary aspergillosis.⁷ Thus, L-AmB can be expected to have the ability to accumulate and achieve fungicidal concentrations at the site of fungal infection.¹⁴

Previous investigators have concluded that the administration of L-AmB in experimental animals results in a higher C_{max} value in blood but in lower tissue concentrations compared with CAB.^{7,15} In uninfected mice, the drug concentration in the lung was found to be lower.^{5,15} The lung is not an organ of the reticulo-endothelial system such as the liver or spleen, and L-AmB binds to lung tissue at a low rate.

Some studies have determined the distribution of AmB in autopsied human lung and other organs.^{3,8,11,16–18} The highest

AmB concentrations have been found in liver and spleen, whereas lesser amounts of the drug have been found accumulated in kidney and lung. For example, the mean AmB tissue concentrations in the liver, spleen, kidney, and lung from eight patients treated with CAB (total doses of 101–2 688 mg) were 80.2, 50.9, 16.4, and 11.3 μg/g, respectively.¹⁶ More recently, the mean concentrations from seven patients given L-AmB (mean cumulative dose was 2 739 mg) were documented as 102.8 μg/g (liver), 60.3 μg/g (spleen), 11.9 μg/g (kidney), and 11.6 μg/g (lung).⁸ However, whether the autopsied lung tissues in these studies were infected or not is unclear.

Demartini et al. reported the concentrations in plasma and lung tissue in 18 patients with lung cancer after a single L-AmB intravenous infusion; lung resection was done at 10–25 h after the infusion.¹⁹ In their study, blood and lung tissue were sampled during the operation and assayed for AmB concentrations. Interestingly, the plasma AmB concentration progressively decreased after completion of the infusion (from 3.4 μg/ml to 1 μg/ml), but the opposite occurred in lung tissue, where the AmB concentration increased with time after the end of the infusion (from 1 μg/g to 2.5 μg/g).

Meanwhile, in the same study, the tissue AmB concentrations were much lower than those of our patient.¹⁹ L-AmB was given to their patients only once at a lower dose (1.5 mg/kg), and no infectious lesion was available in the resected lung. In our study, L-AmB was given daily for 49 days at higher doses (2.8 or 4.7 mg/kg), and the drug concentrations in plasma and tissues were much higher. Some studies have demonstrated that the total dose of L-AmB is correlated with its concentration in the lung.^{17,18} Heinemann et al. reported that with higher cumulative doses of L-AmB (1 200 mg) administered, higher concentrations of AmB were detected in the lung tissue of patients (69.4 μg/g), whereas liver and spleen concentrations were found to remain relatively unchanged.³ These findings suggest that the reticulo-endothelial system, which is at least partly responsible for clearance of liposomes from the circulation, transiently becomes saturated, leading to more drug accumulating in the lung.³ Our result of increased concentrations of AmB detected in the lung tissue of the patient is consistent with these previous findings.

In our study, a much higher concentration of AmB was observed in the infected lung lesion than in uninfected lung tissue. Although the data described here are from only one patient, our results support the hypothesis that L-AmB accumulates in lesions of fungal infection.^{5,13,14} A methodological limitation is based on the impossibility of assessing individual variability, because tissue samples could be collected only once. Still, our results shed light on the process of fungal infection affecting drug accumulation and distribution. The degree to which our results can be generalized to other patients with fungal infections is unclear, but this warrants further examination.

Conflict of interest

There was no financial or personal relationship with other people or organizations that could have inappropriately influenced (biased) this work.

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